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TWO WATER MITE SPECIES (ACARI: HYDRACHNIDIA) FROM KARST SPRINGS NEW FOR THE FAUNA OF CROATIA WITH NOTES ON DISTRIBUTION AND ENVIRONMENTAL PREFERENCES

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One fifth of all described water mite species show clear preferences to spring ecosystems. The main goal of this research was to contribute to the knowledge of water mite assemblages, species richness and abundance in karst springs, with a special emphasis on comparing rheocrene and limnocrene spring ecosystems. The limnocrene spring Modro Oko in the Southern Dalmatia had only one water mite species recorded in the studied period, showing very low abundances, whereas the rheocrene spring of Jadro River in the Central Dalmatia showed both higher species richness (ten taxa recorded) and abundance. First records of two water mite species of the family Hygrobatidae from karst springs situated in the coastline area of Croatia are provided and discussed: *Hygrobates setosus* Besseling, 1942 and *Atractides distans* (K. Viets, 1914). Including these two additional records, a total number of 88 water mite species are recorded from Croatia so far.

Key words: Hygrobatidae, karst springs, rheocrene, limnocrene, new records, Dalmatia

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Čak petina svih opisanih vrsta vodengrinja pokazuje izrazitu povezanost s izvorskim ekosustavima. Glavni cilj ovog rada bio je doprinijeti poznavanju zajednica, bogatstva vrsta i gustoće populacija vodengrinja u krškim izvorima, s posebnim naglaskom na usporedbu limnokrenih i reokrenih ekosustava. U limnokrenom izvoru Modro Oko u južnoj Dalmaciji zabilježena je samo jedna vrsta vodengrinja koja je u istraživanom razdoblju zabilježena s relativno malom gustoćom populacija, dok je u reokrenom izvoru rijeke Jadro u srednjoj Dalmaciji nađeno veće bogatstvo vrsta (10 zabilježenih svojti) kao i veća gustoća populacija. U ovom radu prikazani su prvi nalazi dviju vrsta vodengrinja porodice Hygrobatidae iz krških izvora priobalne Hrvatske: *Hygrobates setosus* Besseling, 1942 i *Atractides distans* (K. Viets, 1914). S dva dodatna nalaza, u Hrvatskoj je zabilježeno ukupno 88 vrsta vodengrinja.

Ključne riječi: Hygrobatidae, krški izvori, reokreni, limnokreni, novi nalazi, Dalmacija

INTRODUCTION

Mites (Acari) are primarily terrestrial animals (Davids & Belier, 1979), but some groups like the true water mites (Acari: Hydrachnidia) have invaded aquatic ecosystems (Krantz & Walter, 2009). Since their freshwater invasion, water mites have developed a high diversity - around 6.000 species are described,

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with experts estimating these numbers to exceed 10.000 worldwide (Davids *et al.*, 2007; Goldschmidt, 2016). Representatives of this group inhabit most types of freshwater ecosystems (Miccoli *et al.*, 2013; Więcek *et al.*, 2013), but are often neglected in environmental research due to perceived difficulties in identification (Proctor, 2007; Goldschmidt, 2016). In Europe today, around 1.000 species are described (Watermite.org, 2016), with the research on this group rapidly growing due to recently published comprehensive identification keys (Davids *et al.*, 2007; Di Sabatino *et al.*, 2010; Gerecke *et al.*, 2016). In Croatia, the first checklist was published by Pešić (2002). Recent research in Croatia focused on the ecology of this group in both lentic and lotic Dinaric karst hydrosystems (Pozojević *et al.*, 2018a; 2018b; 2019).

Springs play an important role in water mite assemblages (Di Sabatino *et al.*, 2003; Gerecke *et al.*, 2018). Among all aquatic invertebrates, they represent the highest ratio of crenophilous and crenobiont taxa, with approximately 20% of all species showing preference to spring habitats (Gerecke *et al.*, 2018). In general, karstic springs differ from springs of other typology in a higher degree of heterogeneity in terms of size, discharge characteristics, and water level fluctuations (Bonacci, 1993; Smart & Worthington 2004; Krešić, 2010).

The goal of this research is to contribute to the knowledge on the distribution of water mite assemblages, species richness and abundance in karst springs, with a special emphasis on comparing rheocrene and limnocrene ecosystems.

MATERIAL AND METHODS

The study area is located in the Dinaric Western Balkan ecoregion (Illies, 1978) of Croatia. Two karst springs with perennial discharge were selected: a river-forming rheocrene, the Jadro spring, and a lake-forming limnocrene, the Modro Oko spring (Glazier, 2009). The Jadro and Modro Oko springs (Fig. 1) are situated in South and Southeast Croatia, respectively, in a region mostly characterized by the Mediterranean, temperate humid climate with dry and hot summers, and mild and rainy winters (Šegota & Filipčić, 2003). The Jadro spring is located in the middle of Dalmatia on the foothills of the Mosor and Kozjak mountains. The spring is situated at an altitude of 35 m a.s.l., and it is part of the Cetina River basin (Loborec *et al.*, 2015). The protected freshwater spring Modro Oko is a significant landscape unit situated on the right bank of the Neretva River in Southern Dalmatia at an altitude of 9 m a.s.l.. It is influenced only slightly by seawater (≤ 4‰) from the Adriatic, infiltering through the main body of the Neretva River, a marine lake (Vlaška) and the Crna Rijeka River (Jasprica & Hafner, 2005; Gottstein Matočec *et al.*, 2006; Žganec *et al.*, 2010).

Each spring was sampled at two separate occasions: Jadro in May 2014 and in January 2015, whereas Modro Oko was sampled in November 2014 and in January 2015. At each sampling occasion, 20 subsamples were collected from each spring, taking all microhabitat types into consideration that made up at least 5% of the total substrate (AQEM Consortium, 2002). The samples from the Modro Oko were taken only within the littoral zone, while the samples from Jadro were taken from all parts of the spring. The substrate was disturbed up to 5 cm in depth. Benthic macroinvertebrates were collected with a hand net (0.0625 m^2 area and 200 μm mesh size) and preserved in 96% ethyl alcohol. Water mites from the samples were subsequently isolated and fixed in Koenike's solution. Adult water mite individuals were determined to species or genus level using general keys by Davids *et al.* (2007), Di Sabatino *et al.* (2010) and Gerecke *et al.* (2016). Deutonymphs were determined to genus level using the key of Tuzovskij (1990). All water mite specimens are deposited at the Department of Biology, Faculty of Science, Zagreb, Croatia.

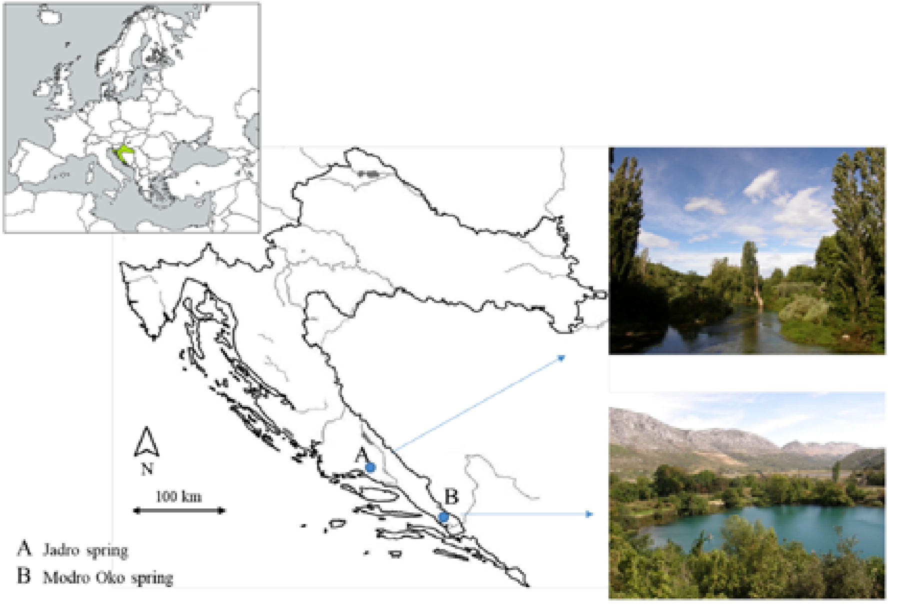

Fig. 1. The location and situation of the two study sites.

The following variables were measured at each spring: air and water temperature, oxygen concentration and saturation (WTW optical oxygen sensor: FDO 925), chemical oxygen demand (COD; potassium permanganate oxidation by Standard Methods - APHA, 2005), conductivity (WTW electrode conductivity measuring cell: TetraCon 925), average current velocity (SonTek FlowTracker), pH (WTW pH electrode: SenTix 940) and alkalinity (titration with 0.1 M HCl with methyl orange used as indicator).

RESULTS AND DISCUSSION

The Jadro spring, because of its rheocrenic nature, is characterized by a considarable current, while in the limnocrene Modro Oko no water current was measurable (Tab. 1). The turbulent water flow in the Jadro rheocrene results in oxygen saturation exceeding 100%, while this value was around 90 % in Modro Oko spring. Between the two respective sampling data, the water temperature was more stable in the rheocrene (oscillation of 0.1°C), when compared with the limnocrene (oscillation of 1.4°C). The variability of the limnocrene water temperature was influenced by the air temperature because of the slow flow of current and direct insolation, while the riparian shadding on the rheocrene was greatly reducing temperature oscillations. The conductivity is in both springs at typical levels for springs with elevated alkalinity due to dissolved calcium carbonate. The sea water infiltration had obviously only a minimum effect on Modro Oko. Both springs are relatively pristine, with little or no inflow of allochthone organic matter and/or nutrients in the groundwater catchment. Slightly higher levels of organics (expressed by chemical oxygen demand, COD) were measured in the standing water of the limnocrene, when compared with the rheocrene.

Tab. 1. Environmental parameters measured at Jadro and Modro Oko springs, Croatia.

	Jadro Spring		**Modro Oko Spring**	
Coordinates	N 43°32′30.0″; E 16°31′07.5″		N 43°05′77.0″, E 17°51′07.0″	
Spring type	Rheocrene		Limnocrene	
Altitude (m a.s.l.)	17		9	
Sampling date	May 18th 2014	January 4th 2015	November 28th 2014	January 3rd 2015
Microhabitat coverage	60% phytal (macrophytes); 30% macrolithal;10% mesolithal	60% microlithal + mesolihtal; 40% phytal (macrophytes)	80% phytal (macrophytes) + argyllal*; 20% mesolithal	70% argyllal*; 30% phytal (macrophytes)
Air temperature (°C)	21.5	10.8	15.1	12.3
Water temperature (°C)	12.7	12.6	13.4	12
Oxygen concentration (mg/L)	11.1	10.77	9.17	9.71
Oxygen saturation (%)	104.2	101.7	87.2	90.2
pH	7.9	7.8	7.52	7.41
Conductivity (μS/cm)	375	448	447	474
Alkalinity (mg $CaCO_3$/L)	190	205	220	232.5
Chemical oxygen demand (O_2 mg/L)	0.67	1.49	4.4	4.65
Current velocity (m/s) - min	0.04	0.48	0	0
Current velocity (m/s) - max	0.24	0.98	0	0
Current velocity (m/s) - average	0.13	0.73	0	0
Water depth at sampling site (cm) - max	60	50	40	70
Water depth at sampling site (cm) - min	25	30	25	5

*argyllal substrate = silt, loam and/or clay

During this research, a total of 102 water mite specimens (92 adults, 7 deutonymphs and 3 larvae) were collected, belonging to 10 species, 7 genera and 6 families (Tab 2). The highest diversity was found at the rheocrene in May 2014 on the mesolithal microhabitat (here also the highest recorded abundance: 200 individuals per square meter). In the limnocrene, during the study period only

Tab. 2. Water mite species and number of specimens recorded at Jadro and Modo Oko springs, Croatia. The calculated abundances per square meter are given in brackets.

Study site:		**Jadro Spring**					**Modro Oko Spring**			
Date:		May 18th 2014			January 4th 2015		November 28th 2014		January 3rd 2015	
Microhabitat sample:		phytal (macrophytes)	macrolithal	mesolithal	microlithal + mesolihtal	phytal (macrophytes)	phytal (macrphytes) + argyllal	mesolithal	argyllal	phytal (macrphytes)
Family	Species									
Hydryphantidae										
	Protzia sp.	1 (1.3)								
Hygrobatidae										
	Atractides sp.	34 (45.3)	5 (13.3)	12 (96)						
	Atractides distans (K.Viets, 1914)		2 (5.3)							
	Atractides gibberipalpis Piersig, 1898	5 (6. 7)	1 (2.7)	4 (32)						
	Atractides nodipalpis (Thor, 1899)	4 (5.3)		3 (24)						
	Atractides pennatus (K. Viets, 1920)				2 (2.7)					
	Hygrobates setosus Besseling, 1942						3 (3.4)		1 (1)	1 (4)
Lebertiidae										
	Lebertia sp.	4 (5.3)	5 (13.3)	2 (16)	1 (1.3)					
Oxidae										
	Oxus setosus (Koenike, 1898)			1 (8)						
Sperchontidae										
	Sperchon sp.	2 (2.7)	2 (5.3)	1 (8)						
Torrenticolidae										
	Torrenticola sp.		1 (2.7)	2 (16)						
Hydrachnidia larvae		3 (4)								
Water mite species richness		6	6	7	2	0	1	0	1	1
Water mite abundance (ind./m^2)		70.7	42.7	200	4	0	3.4	0	1	4

Hygrobates setosus Besseling, 1942 was found, with low abundances not exceeding 4 individuals per square meter.

The rheocrene was characterized by different rhithrobiont taxa such as: *Oxus setosus* (Koenike, 1898), a species usually found in lowland rivers (Di Sabatino *et al.*, 2010), *Atractides distans* (K. Viets, 1914), *A. gibberipalpis* Piersig, 1898, *A. nodipalpis* (Thor, 1899), but also a typical crenobiont species: *A. pennatus* (K. Viets, 1920) (Gerecke, 2003). Following Gerecke *et al.* (2018), rheocrenes should usually be inhabited by assemblages of typical rheocrene species and rhitrobionts, whereas limnocrenes should be characterized by crenobionts not specialized to these habitats and ubiquitous species. So far, *Hygrobates marezaensis* Pešić and Dabert, 2017 from Montenegro and Croatia is the only known typical limnocrenobiont (Pešić *et al.*, 2017). In our study, the rheocrene showed much higher abundances and taxa richness when compared to the limnocrene. *Hygrobates setosus*, the only species found in the Modro Oko, has recently been recorded also in the strong-flowing limnocrene Vitoja in Montenegro (Pešić *et al.*, 2019). This species has only recently been distinguished from *H. nigromaculatus* Lebert, 1879 by Martin *et al.* (2010), and belongs to the *H. nigromaculatus* complex. *Hygrobates nigromaculatus* Lebert, 1879 is usually found in lakes (recorded in Croatia by Pozojević *et al.*, 2019), whereas *H. setosus* is in general inhabiting streams.

Since the first checklist of water mites in Croatia (Pešić, 2002, with 48 species), information on the group was broadened in two occasions in the course of research on water mites of the Balkan Peninsula (Pešić *et al.*, 2010, 2018). These checklists are missing records from Matoničkin & Pavletić (1959) [*Woolastookia rotundifrons* (K. Viets, 1922)] and the doctoral thesis of Lattinger (1988) [*Partnunia angusta* (Koenike, 1893), *Panisus torrenticolus* Piersig, 1898 and *Protzia squamosa* Walter, 1908]. Furthermore, Pozojević *et al.* (2018a) published records of three water mite species new for the fauna of Croatia from lake Torak and Čikola River, and Pozojević *et al.* (2019), in a comprehensive overview for the fauna of reservoirs in the Dinaric ecoregion (Illies, 1978), added an additional 19 species. Including the two additional records given here, a total of 88 water mite species is now recorded from Croatia.

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